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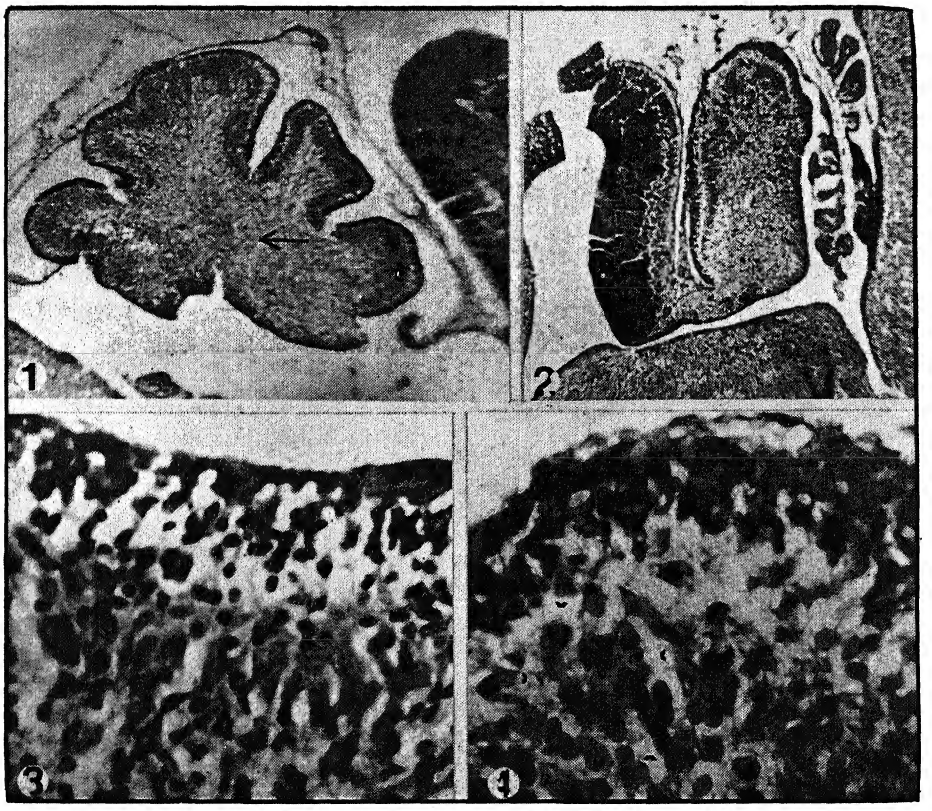

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Prenatal haloperidol induced growth stunting and neuronal alterations in developing rat cerebellum

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Abstract The dopamine (DA) receptor antagonist, haloperidol (HAL) at a dose of 50 mg/kg BW was administered intraperitoneally to pregnant Charles-Foster dams on gestation day 12. An equivalent amount of vehicle (normal saline with 1.5% benzyl alcohol) was given to control dams. This single dose treatment consistently produced stunting of body and brain weight of HAL treated fetuses to roughly 40% of controls and significant reduction of brain and body wieght ratio also. Micromorphological observation of haloperidol treated specimens showed delayed cerebellar development resulting into loss of lobule formation at this stage. Generalised ballooning of both pyramidal and granule cells was observed in molecular and granular layer. The Purkinje cells were not yet differentiated. These findings indicate neuronal degeneration during critical period of cerebellar development and differentiation as well as disturbed migration of neural cells. Therefore, it is concluded that single dose of prenatal haloperidol exposure during brain growth spurt induces not only stunting of the *in utero* body and brain weight but also initial loss of cerebellar neurons which may cause lasting behavioural teratogenicity in rat offspring.

Keywords : haloperidol/prenatal exposure/growth/ cerebellum/rat)

Haloperidol (HAL), a typical antipsychotic drug of butyrophenone group, is frequently prescribed for the treatment of severe manic and schizo-affective neural disorders. It's critical action is to block the dopamine (DA) receptors in general and DA2 subtype particularly[1,2].

There is increasing evidence that prenatal HAL administration during critical developmental stages alter the foetal body and brain weight. Conflicting reports are available on body weight i.e. reduced body weight[1-8], no alterations[9-10] or increased body weight[11]. Reports on reduced foetal brain weight are also available[1,8].

In mice, it was reported that prenatal administration of single high dose of haloperidol (23-35 mg/kg BW) on very beginning of 9th day of gestation induced neural development abnormalities like exencephaly, ectopia of neural tube, dilation of IVth brain

ventricle and retardation of choroid plexus on day 13 of gestation[6,12]. such neuroanatomical deformities were not found in our laboratory except dilation of 3rd brain ventricle when HAL was administered on late 9th day of gestation[7]. On the other hand, HAL exposure on GD 12 or 14 induced not only miniaturization of the brain, but major disruption of structural integrity of the brain, more detected in the cerebral cortex, corpus striatum and hippocampus[7,13]. There is paucity of literature on prenatal HAL exposure and its effects on micromorphological aberrations of cerebellum of rat fetuses. In rodents, experimental studies have indicated that prenatal HAL exposure during critical period of brain development[14] induced neurochemical[3,8-10] (at least dopamine) and structural[7,12,13] alterations in various foetal brain areas especially in dopaminergic rich sites like limbic areas and basal ganglia which may be responsible for long-lasting behavioural dysfunctions of young and adult rat offspring[3,15-17].

In most of the *in utero* experimental studies in rodents, the doses of HAL were low and exposure period was long i.e. covering almost second and/or third week of pregnancy[1,2,8,15,16]. However, no reports are available except that of Jurand and co- worker[12] and a few reports from this laboratory in the recent past[7,13,17] about the effect of a single (high dose) exposure of HAL during gestation period in rats. In view of the non- availability of literature on neuro-micromorphological teratogenicity of single day *in utero* exposure to HAL during critical period of neuronal development, present study has been taken to assess the micromorphological aberrations in cerebellum of rat fetuses.

Inbred Charles-Foster rats (150-200 g) were maintained for 4 weeks prior to experimentation. These rats were maintained at standard laboratory conditions, viz. 24±2°C room temperature, 50±10% relative humidity and 12 h light/12 h dark cycle (lights on at 07.00 hr). All animals were housed in transparent acrylic cages with bedding material of rice bran. The pelleted food and tap water were available *ad libitum* throughout the experiment. Individual nulliparous female and experienced male rats (1:2) were caged together overnight for mating. On next morning (08.00 hr.), mating was inferred by the presence of sperm in vaginal swab and it was designated gestation day (GD) O. Bedding material was changed twice a week throughout the experiment.

Haloperidol decanoate (50 mg/kg BW, Senorm L.A., Sun Pharmaceutical Industries Ltd. Vapi, Gujarat) was administered intraperitoneally once in a single dose at 09.00 hr on day 12 (GD 12) in pregnant rats, (Group A, n = 3). The pregnant control rats were treated similarly with equal volume of vehicle (90% normal saline with 1.5% benzyl alcohol (Group B, n = 3). Both control and treated dams were sacrificed on day 21 gestation (GD 21) by over dose of ether anesthesia. Their brains were quickly removed, blotted dry, weight individually and again examined for any overt malformations and then fixed in 10% neutral formaline. After fixation, the cerebellum was further processed for histological evaluations by staining with H & E.

Table 1 – Effect of single haloperidol exposure on body, brain and brain/body weight ratio of rat fetuses.

	Control Group (n=8)		HAL Group (n=8)		p value	% of reduction
	Mean	S.D.	Mean	S.D.		
Fetal body weight (g)	4.68	0.36	3.01	0.18	0.001	35.70
Fetal brain weight (g)	0.20	0.01	0.11	0.01	0.001	45.00
Fetal brain/body weight ratio × 100	4.23	0.44	3.63	0.20	0.001	40.35

On gross examinations of GD 12 HAL treated fetuses, the scattered replicability of external anomalies of body and limbs were observed as previously reported[7]. There was a significant reduction ($P < 0.001$) in weight of body and brain as well as substantial loss of brain/body weight ratio in experimental rat fetuses as compared with those of controls (Table 1).

In addition to this, micromorphological observation of stunted foetal brain showed retarded development of cerebellum. In control foetal cerebellum, 4-5 well developed lobules were discernable while secondary lobules were under the developmental process (Fig. 1) whereas in treated specimen still there was no sign of even primary lobular development (Fig. 2). In periventricular zone, neuroblasts were condensed to form cerebellar nuclei in the control specimen whereas no such aggregation was observed in the treated specimen.

In both control and treated cerebellum, molecular and granular layers were developed but in HAL treated specimen, the two layers were separated by a plane of trabecular oedematous zone.In the granular layer, cellular density looked to be reduced as compared to the control specimen. On higher magnification, the cellular size in both the layers was observed to be comparatively enlarged due to generalised ballooning of cells. Plenty of cells were observed to be undergoing granular degeneration after initial ballooning (Fig. 3, 4). Gliosis surrounded by oedematous spaces was observed in the granular layer. In both control and treated specimens, Purkinje cell layer was not demarked well because the Purkinje cells were still under the process of differentiation and superficial migration. In control group, the differentiating and superficially migrating Purkinje cells were frequently observed in the granular layer whereas in treated cerebellum, no such cells were observed in the granular layer. All cells in this layer were enlarged, rounded in shape and looked similar indicating delayed differentiation and migration of the Purkinje cells.

This experiment clearly demonstrates that prenatal haloperidol exposure has a highly replicable effect on body and brain weight of rat fetuses. This effect substantially reduces the body and brain weight of fetuses to roughly 40% of controls. The brain weight was more

affected than body weight. Further, the ratio of brain and body weight also indicates significant replicable stunting of both somatic and neural growth in developing fetuses.

Miniaturization of body and brain weight reported so far may be attributed to the relatively prolonged low dose HAL exposure[1-3,9,16]. So far, no literature is available except that of Jurand and co- worker[6,12] on foetal CNS malformations induced by prenatal exposure to single high dose of HAL (23-35 mg/kg) on day 9 of pregnancy in mice. The high doses of other antipsychotic drugs like chlorpromazine (28 mg/kg) were also administered[6,12]. These doses were approximately similar to average therapeutic doses for human. Our selectivity of high dose of HAL corroborates with Jurand and co-workers[6,12] who have reported substantial reduction of foetal weight. Further, the dose given to pregnant rats in the present study corresponded to recommended therapeutic doses in human beings.

The reports from clinical studies indicate that neuroleptics such as haloperidol do not produce congenital malformations[18]. In contrast to this, experimental studies have indicated that prolonged exposure of haloperidol during prenatal period significantly induces intrauterine growth retardation of body and brain even affecting regional brain weight of pups and adults[1-3,8,16]. In pregnant mice, single day exposure of HAL (23 mg/kg BW) on early hour of GD 9 induces CNS malformations including dilation of IVth brain ventricle and reduced choroid plexus[6,12]. Earlier this laboratory has reported that single day exposure to HAL (50 mg/kg BW) on GD 12 not only alters the size of IIIrd brain ventricles and choroid plexus volume but also causes significat modifications in frontol cortex (reduced cortical thickness and neuronal density), corpus striatum (reduction in size and loss of neuronal cells) and hippocampus (altered cytoarchitectural pattern and neuronal loss) of rat fetuses at GD 21[7,13]. But, no such alterations were observed in GD 9 exposed groups because day 9 is the critical stage of morphogenesis when the neural tube is closing. In the present findings, GD 9 HAL exposed fetuses showed negligible effect on development of the cerebellum which was excluded from this study. Short exposure of such a drug could therefore result in teratogenicity whereas chronic treatment could cause embryo lethality[19].

In the present study, poor development of cerebellum and its lobules, delayed differentiation and scattered migration of Purkinje cells as well as degeneration of granule cells suggest that distrubances of organogenesis, neurogenesis and migration of cells occur if HAL is administered during brain growth spurt[14]. This suggests that there might be a susceptible period of Purkinje cell development and differentiation that could be affected by prenatal HAL exposure. Gragg and Phillips[20] reported that ethanol exposure from embryonic day 12 to PND 5 results in a heavy loss and uneven distribution of Purkinje cells in the cortex and retardation of the foliation of cerebellum i.e. 7 to 8 lobules had formed in ethanol treated brains instead of 10 in controls. The results obtained from the present study support the findings of

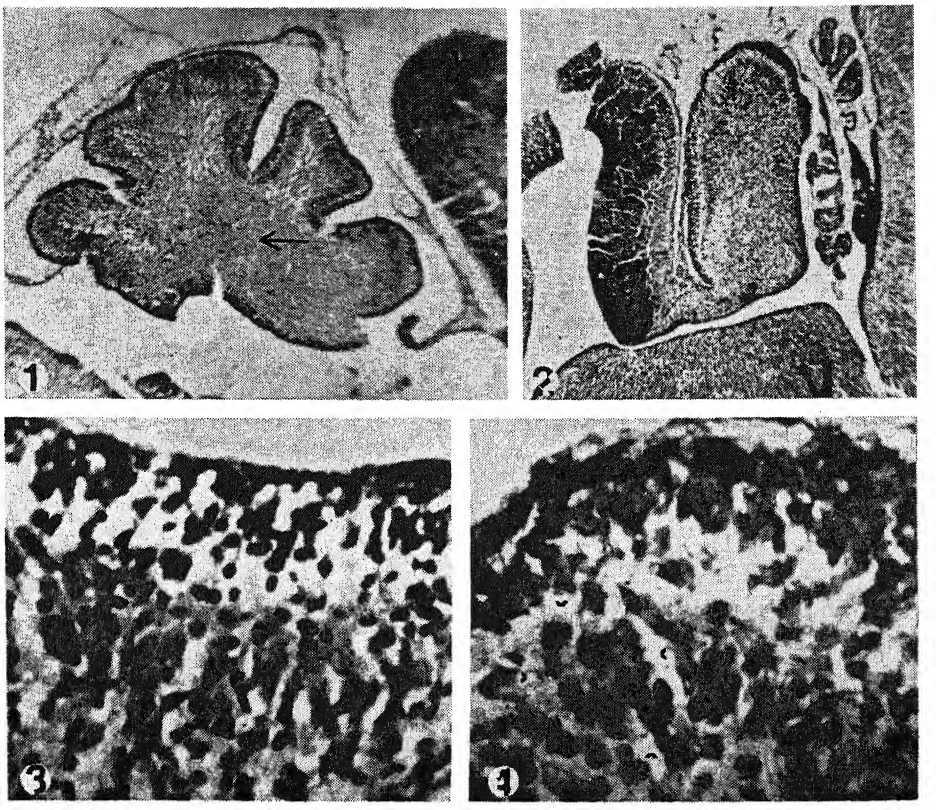

Fig. 1 – Control foetal cerebellum showing aggregation of cells for the formation of cerebellar nuclei (arrow) × 50.

Fig. 2 – Cerebellum showing absence of the development of folia at this stage. No aggregation of cells is visible for the development of cerebellar nuclei × 50.

Fig. 3 – Mulecular and granular layers of control cerebellum × 150.

Fig. 4 – Treated cerebellum showing ballooning and granular degeneration of neuronal cells in both the molecular and granular layers. The two layers are separated by oedematous space which is also infiltrating into the granular layer around the areas of gliosis (arrowheads) × 150.

Gragg and Phillips[20] in loss of Purkinje cells and foliate retardation of cerebellum. Jacobson[21] reported that Purkinje cells differentiate on embryonic day 14 to 17 in rat brain. Altman and Bayer[22] also reported that Purkinje cells are the earliest generated cells (prenatally generated) in the cerebellar cortex while granule cells are generated almost entirely postnatally and neurogenesis continues upto postnatal day 21. It is hypothesized from the present study that exposure period of HAL coincides with Purkinje cells generation, differentiation, proliferation and migration which interfere with neurogensis whereas direct HAL induced cell death may not be ruled out. Thus, early loss in neuronal cell acquisition leads to lasting behavioural alterations in rat offspring since proliferating, migrating or differentating nerve cells are more susceptible than mature cells[23]. In rats, gestation day 12-21 of pregnancy appears to be most vulnerable to the action of neuroactive drugs because this is the critical period of neurogenesis, organogenesis, synaptogenesis, formation of specific neural circuits[24], rapid cell proliferation[1] and functicnal maturation of central dopaminergic system in brain[2]. It has generally been thought that the dopamine system in the rodent brain does not begin to develop prior to day 12 of gestation[1]. Immunocytochemical studies show that dopaminergic fibres from substantia nigra/ ventral tegmental area do not reach the forebrain before GD 15[25] while HPLC studies suggest that dopamine and its metabolite levels are not detectable much before GD 17[26]. Bouthnet *et al*[27] reported that dopamine D2 and D3 receptors were present in the molecular layer of cerebellum. The presence of dopamine in elements of the cerebellar cortex is still disputed. In the present study, it is hypothesized that HAL treatment given on GD 12 may alter the development and differentiation of DA receptors which induces structural demormities of cerebellum in foetal rat brain because at this stage the dopaminergic neurotransmission system is mature enough to play a rather general role in supporting the rapid cell proliferation.

Thus, results obtained from the present study reveal that even a single (high dose) prenatal HAL exposure may induce micromorphological alterations in the developing cerebellum. Hence, clinical use of HAL for treatment of various CNS disorders during human pregnancy should again be questioned.

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References

1. Holson, R.R., Webb, P.J., Crafton, T.F. & Hansen, D.K. (1994) *Teratology* **50** : 125.
2. Zhang, J., Wang, L. & Pitts, D.K. (1996) *Neurotoxicol. Teratol.* **18** : 49.
3. Scalzo, F.M., Holson, R.R., Gough, B.J. & Ali, S.F. (1989) *Pharm. Biochem. Behav.* **34** : 721.
4. Spear, L.P., Shalaby, I.A. & Brick, J. (1980) *Psychopharmacology* **70** : 47.
5. Bhanot, R. & Wilkinson, M. (1982) *Experientia* **38** : 137.
6. Jurand, A. (1980) *Dev. Growth Diff.* **22** : 61.

7. Singh, K.P. & Singh, M. (1999) *Proc. Natl. Acad. Sci. India* **69** : 43.

8. Williams, R., Ali, S.F.; Scalzo, F.M. Soliman,K. & Holson, R.R. (1992) *Brain Res. Bull.* **29** : 449.

9. Rosengarten, H. & Friendhoff, A.J. (1979) *Science* **203** : 1133.

10. Hill, H.F. & Engblom, J. (1984) *Dev. Pharmacol Ther.* **7** : 188.

11. Shalaby, I.A. & Spear, L.P. (1980) *Pharmacol. Biochem. Behav.* **13** : 685.

12. Jurand, A. & Martin, L.V.H. (1990) *Teratology* **42** : 45.

13. Singh, K.P., Singh, M. (2001) *Ind. J. Exp. Biol.* **39** : 223.

14. Dobbing, J. & Sands, J. (1979) *Early Hum. Dev.* **3** : 79.

15. Scalzo, F.M., Ali, S.F. & Holson, R.R. (1989) *Pharmacol. Biochem. Behav.* **34** : 727.

16. Singh, Y., Jaiswal, A.K., Singh, M. & Bhattacharya, S.K. (1997) *Ind. J. Exp. Biol.* **35** : 1284.

17. Singh, K.P., Jaiswal, A.K. Singh, M. & Bhattacharya S.K. (1998) *Ind. J Exp. Biol.* **36** : 1102.

18. Golberg, H.L. & Di Mascio, A. (1978) in *Psychopharmacology : A generation of progress*, ed. Lipton, M.A., Di Mascio, A. & Killam, K.R., Raven Press, New York, p. 1047.

19. Erikson, M. & Yaffe, S.J. (1973) *A. Rev. Med.* **24** : 29.

20. Cragg, B. & Phillips, S. (1985) *Brain Res.* **325** : 151.

21. Jacobson, C.D., Antolick, L.L., Scholey, R. & Uemura, E. (1988) *Dev. Brin Res.* **44** : 233.

22. Altman, J. & BAyer, S.A. (1978) *J Comp. Neurol.* **179** : 23.

23. Marcusson, B.L., Goodlett, C.R., Mahoney, J.C. & West, J.R. (1994) *Alcohol* **11** : 147.

24. Kellogg, C.K. & Guillet, R. (1988) *Transplacental effects on foetal health*, Allen R. Liss, New York, p. 265.

25. Berger, B. & Verney, C. (1984) in *Monoamine innervation of cerebral cortex*, ed. Descarries, L., Reader, T.R. & Jasper, H.H., Allen R. Liss, New York, p. 347.

26. Ribary,U. Scholumpf, M. & Lichtensteiger, W. (!986) *Neuropharmacology* **25** : 1981.

27. Bouthenet, M.L., Souil, E., Martres, M.P. & Sokoloff, P. (1991) *Brain Res.* **564** : 203.

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Synthesis, characterization and biological activities of lanthanum (III) complexes with indole schiff bases

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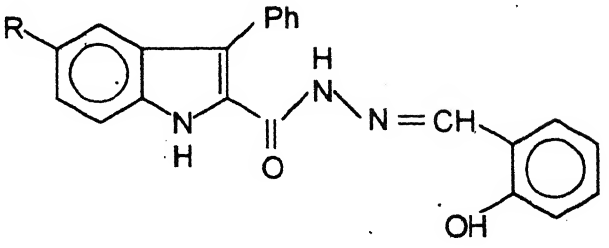
Abstract A few complexes of lanthanum (III) chloride have been prepared by reacting with 3-phenyl-5-substituted indole-2-carboxy hydrazones in alcohol medium. All the complexes are yellow in colour and non-electrolytes in DMF and DMSO. These complexes were characterized using conductivity measurements, IR thermal and ^{1}HNMR spectra. Elemental analysis confirm a stoichiometry 1:2 of the type LaL_2, $Cl.H_2O$. A six coordinate environment for La (III) is proposed.

(**Keywords** : IR/^{1}HNMR/lanthanum (III) complexes/indole schiff bases)

Literature survey reveals that although metal complexes of schiff bases derived from various amines have been studied extensively[1], no report is there on the complexes of La(III) with 3-phenyl-5-substituted indole-2-carboxy hydrazones of salicylaldehydes. The hydrazones are the condensation products of hydrazides and aldehydes or ketones. Some indole-2-carboxyhydrazides have shown good anti-inflammatory and anti-microbial activity[2,3]. The paucity of information on the complexes of the following ligands has infused interest in us to undertake the systematic study of La(III) complexes with these ligands.

Synthesis of Ligands : All the chemicals used were of reagent grade. 3-phenyl-5-substituted indole-2-carboxyhydrazides were prepared according to the method reported earlier[4].



R = Cl, Br, OCH_3

Synthesis of complexes : To an ethanolic solution containing 2,3,5-disubstituted indole-2-carboxyhydrazones (0.02 mol) the La(III) chloride (0.01 mol) was added. The reaction mixture was refluxed for about 2 h and then was added 2 g of sodium acetate. Again the reaction mixture was refluxed for 1h. The mixture was poured into distilled water, the precipitated complex filtered, washed with distilled water and dried in vacuum over fused $CaCl_2$.

La(II) in the complexes was determined gravimetrically as $La_2O_3^5$. Nitrogen was determined by Kjeldhal method. The results of the elemental analysis are summarized in Table 1. The IR spectra of these ligands and the complexes were recorded on a Perkin Elmer- 1000 infrared spectrometer in the region 4000-300 cm^{-1} in KBr discs. The ^{1}HNMR spectra were recorded on a Bruker[1] spectrometer. DSC thermal traces were obtained on a Perkin

Elmer analyser with a heating rate 20°C/min. The molar conductance was measured in DMF and DMSO using an ELICO CM 82T conductivity bridge with a cell of cell constant 1.1 cm^{-1}.

The complexes were yellow in colour and insoluble in common organic solvents. However, they were soluble in DMF and DMSO. The elemental analysis (Table 1) reveals that these complexes have 1:2 stoichiometry of the type $LaL_2Cl.H_2O$, where "L" is deprotonated ligand. The molar conductance values at the concentration 10^{-3}M in DMF are too low to account for any dissociation of the complex in DMF.

IR Spectra : The hydrazones are known to exhibit keto-enol tautomerism and as such they exist in one of the two forms in the complexes[6]. A medium intensity broad band observed around 3260 cm^{-1} and another strong band ~1650 cm^{-1} are assigned to ν(NH) ν(C=O), respectively[7]. It has been established that the formation of inter-molecular hydrogen bonding results in weakening and broadening of the band attributable to-OH vibration and also shifts to lower frequency[8]. In view of this, broad band with fine structure observed in the 2700-2650 cm^{-1} is attributed to the intramolecular H-bonded-OH stretch[8]. This band is absent in the complexes showing that the ligands have reacted with the metal ions *via* deprotonation. The band due to ν(NH)[9] is unperturbed in the complexes.

The band in the region 1650 cm^{-1} for the complexes are assigned to C=O stretch[10]. This band remains unperturbed in the complexes suggest that C=O group does not involve in bond formation.

The band due to phenolic C–O[11] around 1280 cm^{-1} in ligands shows considerably high frequency shift and observed in the complexes around 1370 cm^{-1}. This suggests that oxygen atom of the phenolic –OH group has participated in coordination. The C=N stretch[11] for the ligands observed in the region 1625-1610 cm^{-1} which appeared at 1600-1550 cm^{-1} in the complexes suggesting the coordination between nitrogen of the azomethine group and metal ion. The aquo complexes display a broad and medium intensity band around 3430-3450 cm^{-1} for ν(H_2O) and a broad band at 680 cm^{-1} attributable to $\rho_w(H_2O)$ of the coordinated water molecule[12]. All the complexes exhibit a broad band ~3400 cm^{-1} and 650-665 cm^{-1} indicating the presence of water molecule in the complexes.

Taking into consideration the previous[12] assignments, the bands observed in teh region 490-470 cm^{-1} to ν(La-N) coupled with the ligand vibration and bands observed around 425 cm^{-1} may be attributed to ν(La-O).

DSC Analysis : All the complexes were subject to DSC analysis. The complexes show small and broad endothermic peaks in the region 227°C–297°C due to decomposition of organic moities. There was no change in DSC trace above 297°C indicating the formation of stable La_2O_3. In all the cases the broad peak indicates the amorphous nature of samples.

^{1}HNMR Spectra : The spectra for the ligand exhibit four characteristic resonance signals due to the protons of different environ-

Table 1 – Elemental analysis and IR data of Lanthanum (III) complexes with Indole Schiff Bases.

Complex No.	Empirical Formula	Analytical data					IR data						
		%La	%H	%C	%N	%Cl	ν(OH)	ν(NH)	ν(C=O)	ν(C=N)	ν(C-O)	ν(M-N)	ν((M-O)
1.	$(C_{22}H_{15}N_3O_2Cl)_2$ La.Cl.H_2O	17.00 (17.00)	1.50 (1.50)	27.50 (2723)	4.20 (4.20)	3.55 (3.50)	3402	3292	1660	1614	1372	487	423
2.	$(C_{22}H_{15}N_3O_2Br)_2$ La.Cl.H_2O	17.01 (17.07)	1.32 (1.37)	24.62 (24.49)	3.80 (3.85)	3.20 (3.25)	3402	3286	1659	1613	1370	480	423
3.	$(C_{23}H_{18}N_3O_3)_2$ La.Cl.H_2O	17.0 (17.13)	1.80 (1.81)	28.95 (28.73)	4.20 (4.23)	3.50 (3.57)	3422	3307	1650	1615	1363	472	425

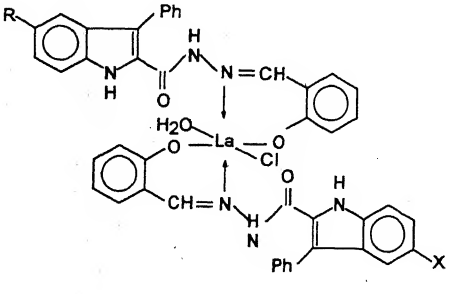
ments. The signals at 13.2 ppm and 9.01 ppm are attributed to *o* - hydroxy and azomethine protons, respectively. A sharp multiplet observed at 6.0 to 7.8 ppm is attributed to the phenyl protons. While methylene protons are observed around 2.4-3.0 ppm.

In the ^{1}HNMR spectra of complexes the following changes were observed.

The azomethine proton signal appears around 8.9 ppm. The down field shift due to deshielding of azomethine protons suggest the coordination of CH=N group through nitrogen.

The signals due to - OH protons disappear in all the complexes indicating that -OH group reacts to La(III) moiety *via* deprotonation. No change in the signals due to phenyl protons was observed in the complexes.

In conclusion, the IR, ^{1}HNMR, conductance and analytical data suggest that coordination has taken place through azomethine nitrogen as well as phenolic oxygen. The presence of coordinated water molecules in all these complexes is confirmed by the observation of a broad band around 3400 cm^{-1}. These facts indicate a hexa-coordination around La(III) in the complexes formed. All these observations project the following probable structure for these complexes.



R = Cl, Br, OCH_3

Biological activity : Indole ring system itself is known for its diverse biological properties. The development of resistance[13,16] among the various pathogenic organisms towards the antibiotics has stimulated the invention of newer antimicrobial agents. Synthesis and the study of antimicrobial property of various indole derivatives have been reported[17,18]. In the present investigation, we have screened the ligands and their complexes for the antibacterial and antifungal activities. The common microorganisms used are *Staphylococcus aureus* as gram positive organism (cocco) antimicrobial activity against. *E.coli* and *S. aureus*. It is evident from the result that complexes containing chloro and bromo substituents at 5' position of indole moiety showed activity against all the organisms tested.

All the compounds tested for their antibacterial activity against *S. aureus* and *E.coli* All the ligands showed weak inhibition when compared to that of complexes. The complexes showed weak inhibition when compared to standard gentamycin, which shows 22mm inhibition against the same organism. Hence, the presence of metal increases the diffusion of the drug solution in the media.

References

1. Holm, R.H. and O' conner, M.J. (1971) *"The stereochemistry bis-chelate metal (II) complexes", Progress in Inorganic Chemistry* (Ed. S.J. Lippard), Willey Interscience, New York, **14** : 241.

2. Hiremath, S.P. Mruthunjaya Swamy, B.H.M. Klasker, N.J. & Kakkeri, R.H. (1990) *Gulbarga University Journal (Science & Technology)*, **38** : 112.

3. Hiremath, S.P. Mruthunjaya Swamy, B.H.M. Purohit, M.G., Kanta, R.C. & Seshayyan, R.J. (1978) *Karnataka Univ. Science.*

4. Hiremath, S.P. Mruthunjaya Swamy, B.H.M. Purohit, M.G. (1978) *Indian J. Chem.* **16B** : 789.

5. Vogel, A.I. (1964) *"A Textbook of Quantitative Inorganic Analysis"*, ELBS 3rd Edn. Longman Gree, London.

6. Biradar, N.S. Havinale, B.R. (1976) *Inorg. Chem Acta.*, **17** : 157.

7. Rajshekhar, G., Shantaveerappa, B.K., Angadi, S.D., Kukarni, V.H. & Mruthunjaya Swamy, BHM (1998) *Asian J. Chem.* **10** : 306

8. Freedman, H.H. (1961) *J.Am. Chem Soc.* **83** : 2900.

9. Ibrahim, K.M., Rakha, T.H. Abdullah, A.M. & Hassanian, M.M. (1993) *Indian J. Chem.* **32A** : 361.

10. Hiremath, A.C., Halli, M.B., Huggi, N.V. (1984) *J. Indian Chem. Soc.* **61** : 191.

11. Biradar, N.S., Kulkarni, V.H. (1971) *J. Inorg. Nucl. Chem.* **33** : 2451.

12. Nakamoto, K. (1978) *Infrared and Raman Spectra of Inorganic and Coordination Compounds,* 3rd Edn., Wiley Inter-science, New York.

13. New, H.C. (1973) *Newer Antibiotics, Diseases-a-month,* 1-6.

14. Watnase, T. (1966) *Infections Drugs Resistance, J. Med.*, New England, **275** : 888.

15. Gandner, P. & Smith, D.H. (1964) *Ann. Int. Med.* **1** : 17.

16. Hatrical Jevous. M. (1964) *British Med. Journal* **1** : 124.

17. Gadaginmath (1974) *Ph.D. Thesis,* Karnataka University.

18. Hiremath, S.P., Purohit, M.G. & Sirsi, M.J. (1974) *Karnataka University Journal,* **19** : 208.

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X-ray K-absorption spectral studies of copper (II) mononuclear, binuclear and trinuclear complexes

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Abstract X-ray K-absorption spectral studies of some copper (II) mononuclear, binuclear and trinuclear mixed-ligand complexes have been carried out using Cauchois type spectrograph of 0.4 m radius. The observed X-ray absorption parameters, e.g., chemical shift and edge-width have been used to explain the coordination in the complexes. A linear dependence between magnetic moment and chemical shift has been observed. The chemical shifts show a parabolic dependence on the effective nuclear charge, Z_{eff}, of copper atom in these complexes. Also a linear correlation has been obtained between the bonding parameter (namely, α_1) and percentage covalency. This bonding parameter may be used for the estimation of covalency of the M-L bond in the other complexes.

(**Keywords** : X-ray absorption/copper complexes/chemical shift/edge-width/effective nuclear charge/percentage covalency)

X-ray absorption spectral studies have proved to be a useful technique[1-5] in the characterization of metal complexes. Though interpretative procedures for the absorption edge features are developing gradually with the new studies, even then the X-ray absorption spectra provide a good clue regarding the electronic structure and nature of the chemical bonding in coordination complexes[6].

It is well known[7,8] that X-ray absorption discontinuity is very sensitive to the physico-chemical conditions of the absorbing atom, conveys very useful information about the ligand field symmetry, effective charge distributiuon of central atom etc. Investigations[9-11] on the edge shift studies in X-ray absorption spectra indicate that the chemical shift (ΔE) is not only dependent on valency but several other factors *viz.*, coordination number[12], ionicity[13], nature of the chemical bond and effective nuclear charge[14,15] etc. Though different chemical parameters, e.g., ionicity, coordination number, stereochemistry etc. have been shown to be correlated[16-18] with the chemical shift (ΔE), yet satisfactory relationships between chemical

Table 1 – Chemical shifts and other X-ray absorption parameters for copper (II) complexes.

Complex No.	Complexes	Position of the edge λ_K(mÅ)	$E_{K\,edge}$ (eV) (±0.2 eV)	Edge-shift ΔE(eV) (±0.2 eV)	Edge-width (eV) (±0.2 eV)	Effective nuclear charge Z_{eff} (electrons/ atom)	Covalency (%)	μ^{**}_{eff} (B.M.)
I	$[Cu(bipy)(Cl)_2]$	1379.54	8987.4	7.0	14.9	0.79	55.5	1.86
II	$[Cu(imH)_4(Cl)_2]$	1379.43	8988.1	7.7	13.6	0.84	54.0	1.90
III	$[(Cl)_2(imH)_2Cu(imH)_2Cu(bipy)(Cl)_2]$	1379.74	8986.1	5.7	16.1	0.68	60.2	1.41
IV	$[(Cl)_2(bipy)Cu(imH)_2Cu(imH)_2Cu(bipy)(Cl)_2]$	1379.81	8985.6	5.2	16.4	0.65	62.8	1.37

* We have used conversion factor λ(mÅ)=λ(XU) × 1.0021017, ** Values taken from reference (38).

shift (ΔE) and these parameters are still to be established.

In the present work, we have investigated the copper (II) complexes as listed in Table 1. These complexes are mononuclear, binuclear and trinuclear metal complexes which are important because of their magnetic, optical and conduction properties[19,20]. The mutual interaction between various metal centres present in such complexes is also of special interest[21]. The purpose of the present investigation is to observe the K- absorption spectra of Cu(II) in mixed-ligand mononuclear, binuclear and trinuclear complexes and to find the dependence of the edge shift (ΔE) on effective nuclear charge (Z_{eff}). Earlier, Mande and Kondawar[22], Sarode *et al.*[23] and Murugesan *et al.*[24] have observed that ΔE shows a parabolic dependence on the Z_{eff} in transition metal oxides. Justification for linear fit for small Z_{eff} values also exists in the literature[25,26].

The aim of the present study is to test this parabolic dependence of chemical shift on effective nuclear charge as well as to study the correlation between chemical shift and magnetic moment. We have also proposed a correlation between the bonding parameter (namely, α_1, defined later) and the covalency of the metal-ligand bond.

The spectra of copper complexes given in Table 1, were recorded on Kodak X-ray photographic film employing 0.4m Cauchois type bent crystal spectrograph. The $\bar{2}01$ planes of a well tested mica crystal was used as reflecting planes. A sealed Seifert X-ray tube with tungsten target operated at 17kV and 55mA was employed as the source of continuous radiation. The absorbing screens were prepared by spreading uniformly fine powder of the samples on 1 cm^2 area of cellphane adhesive tape. The thickness of the absorbing screens was adjusted by several trials to obtain maximum contrast and the best spectra.

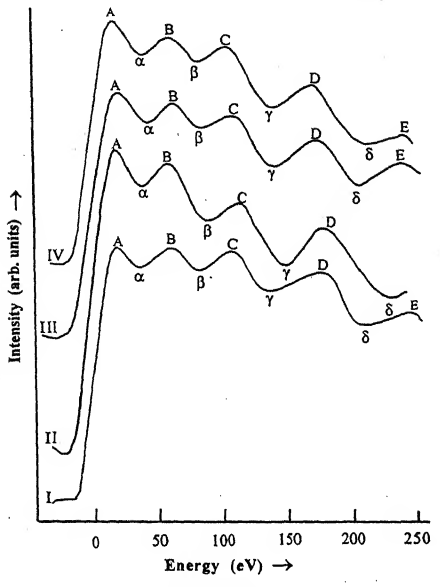

Fig. 1 – Shape and EXAFS of the copper K-absorption discontinuity in some of its complexes. I : $[Cu(bipy)(Cl)_2]$ II: $[Cu(imH)_4(Cl)_2$, III: $[(Cl)_2 (imH)_2 Cu(imH)_2 Cu(bipy)(Cl)_2]$ IV : $[(Cl)_2 (bipy) Cu(imH)_2 Cu(imH)_2 Cu(bipy)(Cl)_2]$

The intensity measurements were carried out on a Carl-Zeiss GII microdensitometer at a magnification of 10x. The average dispersion obtained on the films was about 12.4XU/mm, i.e., 0.63eV mm^{-1}. The general experimental details have been given in our earlier paper[27].

The exposure time for the compounds was varied from 1 to 3 h. The absorption curves were averaged over at least 9 measurements and the effects due to finite grain size of the photographic film were smoothened. The position of the absorption edge was determined from the inflection point on these photometer records. The energy features were calculated by considering $L\beta_4$ and $L\eta$ lines of tungsten as reference lines and were taken from the tables of Cauchois and Senemaud[28].

The profiles of the X-ray K-absorption discontinuity of the complexes under study, obtained from a number of microphotometer traces of several spectra for each sample are shown in Fig. 1.

In all the absorption edges the inflection point was taken as the half maxima on the absorption curve and used as reference point for the measurements of the positions of maxima (denoted by A, B, C, D, ...) and the minima (denoted by α,β,γ,δ.....) of the extended X-ray absorption fine structure (EXAFS). The position of the absorption edges E_K, edge-shifts ΔE (eV) and edge widths are given in Table 1. The maximum error involved in the measurement of energies, as determined statistically is ±0.2 eV for peaks upto 50 eV and ±0.5 eV for peaks of higher energy.

The effective nuclear charges Z_{eff} (electrons/atom) on the copper atom in the complexes under present study, determined by employing the semi-empirical method of Gianturco and Coulson[29] are also given in Table 1.

The percentage covalency of M-L bonding is estimated in the complexes by using Clementi's[30] result for shifts in 1*s* orbital energies in different oxidation states of copper atom and a theoretical graph is plotted with percentage covalency of M-L bonding in all the four complexes. The values of percentage covalency are also given in Table 1.

It is now well established[31-33] that the fine structure within the K-absorption discontinuity and in its close vicinity in a metal

Table 2 – Positions of EXAFS maxima and minima in eV for copper (II) complexes.

Structure	n	Complex Number			
		I	II	III	IV
A	0	17.8	16.7	14.9	13.5
α	1	38	42	40	39
B	2	58	64	60	58
β	3	82	87	82	81
C	4	105	117	109	105
γ	5	133	146	136	135
D	6	174	181	169	170
δ	7	204	230	199	204
E	8	244	-	232	240

corresponds to the transition of an electron from K ($1s$) shell of the absorbing atom to the unoccupied levels of p symmetry situated just beyond the Fermi level, and to any such hybrid levels in the conduction band which may have p admixture. The study of the shape of the K-discontinuity thus helps partially, in understanding the band structure of the metal.

The compounds under present investigations, display the arctan form of the absorption edge arising due to the allowed transition $1s \rightarrow 4p$[34].

On compound formation, usually the interatomic distance increases and dehybridization sets in. As a result, the $4p$ band becomes narrower and no more admixes with the $3d$ band. Now the $1s$ electron misses the $3d$ band and a possible energy gap between $3d$ and $4s$ bands before it finds an empty $4p$ state. Transition from $1s$ to $4p$ levels thus, give rise to shift of K-edge in the complexes under present study towards the high energy side. The kink in the pure copper metal observed by many workers, thus disappears. This is suggestive of the copper acting as a cation in all the complexes studied[35].

It is well known that mixed valency systems exhibit splitting of the X-ray absorption edge. None of our complexes show any splitting of the K-edge in the intensity *vs.* energy curves shown in Fig. 1, thus indicating that in each of the complexes, the copper atom exists in a single valency state. The chemical shifts of copper complexes in the present investigations are given in Table 1. The values of shift lie in the range 5.2 to 7.7 eV. Since the chemical shifts in the present work are less than 10 eV, it indicates that the copper in all the complexes exists in oxidation state +2 in the present investigation.

In the earlier studies[36] chemical shifts of 4.3, 5.3 and 6.8 eV have been reported in the compounds CuO, $CuCl_2.2H_2O$ and $CuSO_4.5H_2O$ respectively, which are all +2 oxidation state compounds. Taking this into consideration and the range of the values of chemical shifts as observed (5.2 to 7.7eV) in the four complexes studied in the present investigations, it can be inferred that the oxidation state for copper in the present compexes is also +2.

The order of values of chemical shift of the complexes is as follow:

II > I > III > IV

The complexes I and II are mononuclear complexes corresponding to one unpaired

Table 3 – Values of wave vector k ($Å^{-1}$) for the EXAFS maxima and minima for copper (II) complexes.

n	$k(Å^{-1})$			
	Complex no. I	Complex no. II	Complex no. III	Complex no. IV
0	2.16	2.09	1.98	1.89
1	3.16	3.35	3.24	3.20
2	3.90	4.10	3.97	3.90
3	4.64	4.78	4.64	4.61
4	5.25	5.54	5.35	5.25
5	5.91	6.10	5.98	5.96
6	6.76	6.90	6.66	6.68
7	7.32	7.78	7.23	7.32
8	8.01	–	7.81	7.94

spin in copper (II). The structure of $Cu(imH)_4(Cl)_2$ is presented in Fig. 2.

In both the $(Cu(imH)_4(Cl)_2$ and $Cu(bipy)(Cl)_2$, the central metal copper ion is surrounded by nitrogen and chlorine. The chemical shift for both these complexes are higher as compared to complexes III and IV, which are binuclear and trinuclear complexes. In binuclear complexes, two copper centers exist. The two nitrogens become common to both the copper centres. In this way, their contribution to copper centres is halved as compared to that for mononuclear complexes I and II, thereby reducing the effecgive charge on the copper centers and resulting in lowering of the chemical shift value in case of binuclear complex. Similar arguments apply to trinuclear complex where contribution to copper centers is still reduced and hence lower value of chemical shift is observed.

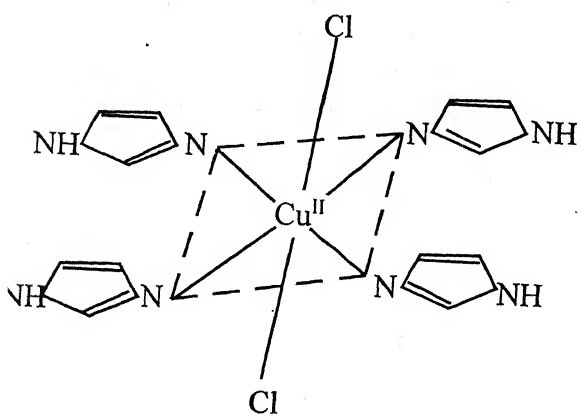

Fig. 2 – The structure of $Cu(imH)_4(Cl)_2$ complex.

The slight difference between the chemical shift values amongst the mononuclear complexes I and II may be attributed to the fact that Cu-imidazole bond being more ionic as compared to Cu-bipyridyl bond, gives a slightly higher value of chemical shift in the case of complex II as compared to complex I. Such a variation of edge-shifts in mononuclear and binuclear complexes of Schiff base of copper (II) complexes has been observed earlier[37].

Chemical studies[38] have been carried out earlier by Patel and Pandeya on these complexes. They have shown that the complex $[Cu(imH)_4(Cl)_2]$ is tetragonally distorted octahedral complex with the imidazole ligand in the z-y plane and chloride ions in the axial positions as shown in Fig. 2 above corresponding to one unpaired spin in copper (II). The two present mononuclear complexes show normal magnetic moments as reported by Patel and Pandeya[38] and the values are 1.90

Table 4 – EXAFS paramters for Cu(II) complexes.

Complex No.	ΔE (eV)	R* (A)	$(R_1 - \alpha_1)$	α_1 (A)	Covaleny (%)
I	7.0	2.51	2.13	0.38	55.5
II	7.7	2.52	2.09	0.43	54.0
III	5.7	2.58	2.23	0.36	60.2
IV	5.2	2.57	2.26	0.32	62.8

*Bond lengths determined by Levy's method.

and 1.86 BM respectively for the complex II and I included in Table 1. The binuclear complex III, on the other hand, shows a subnormal magnetic moment of 1.41 BM per copper atom indicating an antiferromagnetic spin-spin interaction between the imidazole-bridged copper centers. The trinuclear complex IV also shows a subnormal magnetic moment 1.37 BM, probably due to triangular planar structure having all the three Cu(II) centres at corners, again suggesting an antiferromagnetic spin-spin interaction between the bridged copper pairs.

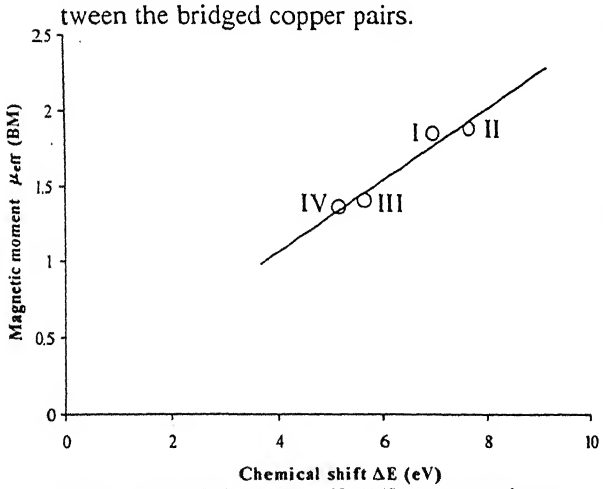

Fig. 3 – Plot of chemical shift (ΔE) *vs.* magnetic moment μ_{eff}

A comparison of μ_{eff} values with the chemical shift values is shown in Fig. 3. A linear variation between these two quantities is observed. The order of variation of μ_{eff} is also as follows :

$$II > I > III > IV$$

which is also the sequence of chemical shift variation. The order of the chemical shifts can

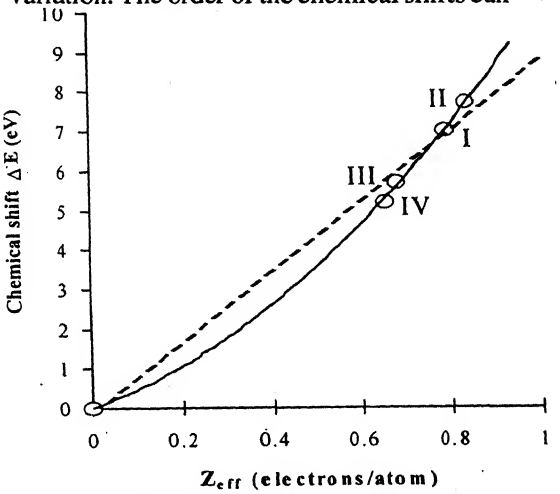

Fig. 4 – Plot of effective nuclear charge, Z_{eff} on copper vs. edge-shift ΔE, for Cu(II) complexes. Dotted line represents regression analysis for linear plot and solid line curve represents the least square fit.

thus be correlated with the order of magnetisation of the complexes. As the magnetic nature is a measure of the free unpaired spin of the electrons in a molecule, such a correlation of the observed chemical shift with the μ_{eff} is expected.

A perusal of Table 1 indicates that the edge widths follow the sequence:

$$IV > III > I > II$$

i.e., the complex IV is more covalant and the complex II least, This is in accordance with the observed variation of chemical shifts. Hence our edge width data also support the relative order of ionicity of the complexes as determined by chemical shift values.

An electron pair is evenly distributed in pure covalent bond between two atoms. If the atoms have different electro- negativities the bond becomes partly ionic and the electrons in the bond have different probabilities of being in the neighbourhood of two atoms. The effective charge is used as a quantitative measure of this tendency.

Various methods are known for determining the effective nuclear charge, Z_{eff}[29,39,40]. In the present work, we have estimated Z_{eff} from the measured chemical shift by using the semi-empirical method of Coulson and Gianturco by employing the procedure outlined by Nigam and Gupta[14]. The estimated values of Z_{eff} are given in Table 1, which shows that the Z_{eff} on the copper atom in the complexes under present investigations are found to be in the range 0.65 to 0.84 electrons/atom.

In the literature, chemical shift (ΔE) has been correlated with effective nuclear charge (Z_{eff}). This has been observed by many workers[22-24] both for binary and ternary systems and for metal complexes. Sarode *et al.*[23] and Murugensen *et al.*[24] observed that in the case of transition metal oxide, ΔE exhibits a parabolic dependence on Z_{eff}. Earlier, Mande and Kondawar observed linear dependence between ΔE and Z_{eff}. In Fig. 4, we have plotted a graph between computed Z_{eff} and experimental ΔE for all the four copper (II) complexes studied.

An attempt has been made to test the probable correlation between ΔE and Z_{eff}. Firstly, the data have been subjected to a linear regression analysis (dotted line in Fig. 4). Inspite of a higher correlation coefficient (0.9876), the observed data are not fitting properly in the linear fit. Hence, the possibility of linear correlation between ΔE and Z_{eff} has been ruled out and as an alternative, the data have been fitted to the expression:

$$\Delta E = a + b\,(Z_{eff}) + c\,(Z_{eff})^2 \qquad (1)$$

The best least square fit is obtained by eqn. (1) with $a = -0.003$, $b = 4.06$ and $c = 6.16$ and standard deviation = 0.076.

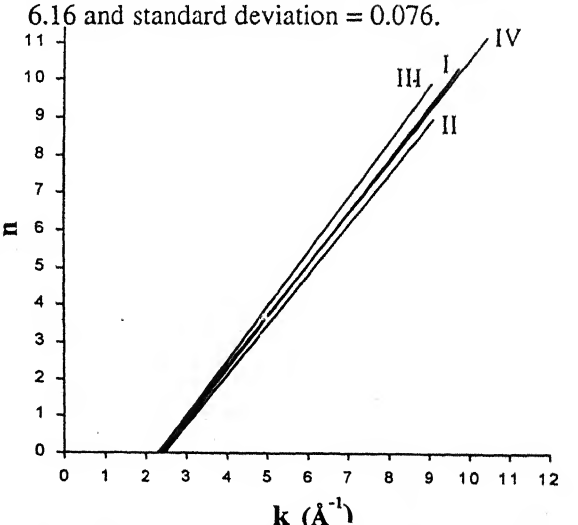

Fig. 5 – Plot of wave vector k ($Å^{-1}$) *vs* n for maxima and minima.

The goodness of the fitted curve is further verified by χ^2 test. χ^2 value is found to be 0.002 whereas tabulated value is 9.488 for 4 degree of freedom at 5% level of significance. The tabulated value is much larger than the estimated value, indicating thereby that the parabolic curve fitted to eqn. (1) is good and is shown in Fig. 4.

Earlier Mande and Kondawar[22] have observed a linear fit between ΔE and Z_{eff}. Later on Khadikar *et al.*[41] observed that linear fit is valid only upto small Z_{eff}.

Justification for the linear fit given by Siegbahn[25] and Carver *et al.*[26] is valid only in the classical limit. In quantum mechanics, the difference in potential felt by an electron in the valency level in a compound and the reference metal should be calculated from a perturbation expansion which would lead to terms of higher power in Z_{eff}. It is therefore, not justified to terminate the expansion on the linear term. The present observation that the Z_{eff} term becomes important at its higher values and a parabolic dependence is obtained between ΔE and Z_{eff} which can be given by eqn. (1) appears justified.

The parabolic nature of ΔE and Z_{eff} plot reported here is similar to that found by earlier workers[23,24]. The present study therefore supports the parabolic dependence of ΔE on Z_{eff}.

Earlier workers[42,43] have proposed various methods to obtain useful structural information from EXAFS data. Lytle[42] have developed a simple graphical method to estimate the average metal-ligand bond distances surrounding the central absorbing metal ion. Further they have shown

$$\left(\frac{1}{2}+n\right)\pi = 2k\,(R_1 - \alpha_1) + 2\beta_1 \qquad (2)$$

by considering the back-scattering from only the first coordination shell to be the dominating one in the case of EXAFS. In above expression R_1 is the radius of the first coordination shell, α_1 is the measure of the chemical bonding known as the bonding parameter, β_1 is the back scattering phase shift and n is the position of maxima and minima in the absorption curve. The value k is obtained from the equation

$$k = (0.263\ E)^{1/2} \qquad (3)$$

where E is the energy of the peaks in the EXAFS. Estimated k values are given in Table 3.

In the plots of n *vs.* k, the lines for different complexes are very close to each other, the individual points have not been shown on the curves in Fig. 5. It is important to not that n *vs.* k curves remain almost the same irrespective of the choice of the zero energy point.

As the absorption edges are fairly similar to each other as shown in Fig. 1, it is obvious that the choice of k as the zero energy point, which corresponds to the vaccum level, appears justified in the case of four copper

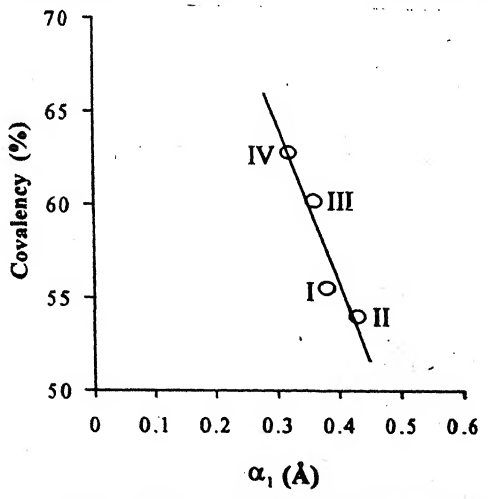

Fig. 6 – Plot between bonding parameter α_1 *vs.* covalency (%).

complexes under present study. This observation is in line with the one observed by Shulman *et al*[44] and Lee and Beni[45], according to which arbitrary assumptions about the zero of energy should not cause any appreciable error. To estimate average interatomic distances and bond lengths, we have used Levy's[46] method.

The values of $(R_1-\alpha_1)$ for Cu(II) complexes are estimated from the slope of curves in Fig. 5 plotted between *n vs. k* and is given in Table 4. We have computed α_1 by substituting the bond diatances (R_1) obtained from Levy's method. The Levy's method is based on the estimation of the near neighbour distances and the data of X-ray absorptions are very reliable in the 0 to 100 eV region. Earlier also, we have reported[27,37] from our laboratory, data on various types of samples with fair degree of accuracy. In Fig. 6, we have plotted a graph between the values of parameter α_1 and the percentage covalency for the copper complexes studied in the present investigations. It shows that the parameter α_1 decreases with increase in the percentage covalency of the metal-ligand bond. Thus, the bonding parameter α_1 may be used for the estimation of percentage covalency of the M-L bond.

No electron absorption data is available in the literature for these samples. However, X-band e.p.r. studies have been carried out by Patel and Pandeya[38]. In the e.p.r. studies reported[38] at room temperature for [(Cu(bipy)(Cl_2)], the e.p.r. spectrum is characteristic of a pseudo-tetrahedral copper (II) complex. The e.p.r. spectrum of the binuclear complex is distinctly different from the spectra of the two mononuclear parent compounds, and is characteristics of a triplet state (s=1) for copper (II). Also for the IV complex i.e., [(Cl_2)(bipy)Cu(im$H)_2$Cu(im$H)_2$Cu(bipy)(Cl_2)], the e.p.r. spectrum shows a triplet state (s=1) for copper (II). The e.p.r. studies carried out by Patel and Pandeya provide Cu-Cu distance (i.e. the distance between two Cu centres) for complex III and IV respectivly to be 6.01 and 7.17Å. In our present studies, we have reported the average metal to ligand distances from the central metal ion i.e., copper. Our values lie in the range 2.51-2.58Å for these complexes. Thus our reported bond distance values provide information on the local environment around the central metal ion copper whereas e.p.r. studies provide distance between two different copper centres in binuclear and trinuclear complexes.

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References

1. Agarwal, B.K. (1991) *X-ray Spectroscopy*, Springer-Verlag, Berlin.
2. Meisel, A., Leonhardt, G.L. & Szargan, R. (1989) *X-ray Spectra and Chemical Binding*, Springer-Verlag, Berlin.
3. Bonnelle, C. & Mande, C. (eds.) (1983) *Advances in X-ray Spectroscopy*, Pergamon Press, New York.
4. Teo, B.K. (1986) *EXAFS : Basic Principles & Data Analyais*, Springer-Verlag, Berlin.
5. Joshi, S.K., Shrivastava, B.D. & Deshpande, A.P. (eds.) (1998) *X-ray Spectroscopy and Al-*

lied Areas, Narosa Publishing House, New Delhi.

6. Kau, L.S., Spira-Solomon, D.J., Penner-Hahn, J.E., Hodgson, K.O. & Solomon, E.I. (1987) *J. Am. Chem. Soc.* **109** : 6433.
7. Cauchois, Y. (1948) *Les Specta de Rayons X et al Structure electronique de la Matiere,* Gauthier-Villars, Paris.
8. Nagel, D.J. & Baum, W.L. (ed.) Azaroff, L.V. (1974) *X-ray Spectroscopy,* McGraw-Hill, New York.
9. Kunzl, V. (1932) *Coll. Trav. Chem. Tchecosl.* **4** : 213.
10. Agarwal, B.K. & Verma, L.P. (1970) *J. Phys. C.* **3** : 535.
11. Srivastava, U.C. & Nigam, H.L. (1973) *Coord. Chem. Rev.* **9** : 276.
12. Srivastava, U.C. (1972) *Nuovo Cimento.* **B 11** : 68.
13. Dey, A.K. & Agarwal, B.K. (1971) *Nuovo Cimento Lett.* **1** : 803.
14. Gupta, M.K. & Nigam, A.K. (1972) *J. Phys. F.* **2** : 1174.
15. Mande, C. & Apte, M.Y. (1981) *Bull. Mater. Sci.* **3** : 193.
16. Pauling, L. (1932) *J. Am. Chem. Soc.* **54** : 3570.
17. Srivastava, U.C. & Nigam, H.L. (1970) *Ind. J. Pure and Appl. Phys.* **91** : 63.
18. Khadikar, P.V. & Pandharkar, S.P. (1986) *Nuovo Cimento.* **D 8** : 33.
19. Patel, R.N. & Pandeya, K.B. (1995) *Nat. Acad. Sci. Lett.* **18** : 103.
20. Pandeya, K.B.& Patel, R.N. (1992) *Nat. Acad. Sci. Lett.* **15** : 267.
21. Interrante, L.V. (1974) (ed.) *Extended interaction between Metal lons, American Chem. Soc.* Washington D.C.
22. Mande, C. & Kondawar, V.K. (1976) *J. Phys.* **C 9** : 1351.
23. Sarode, P.R., Ramaseshan, R., Madhusudhan, W.H. & Rao, C.N.R. (1979) *J. Phys. C : Solid State Phys.* **12** : 2439.
24. Murugesan, T., Sarode, P.R., Gopalkrishnan, J. & Rao, C.N.R. (1980) *J. Chem. Soc. Dalton Trans.* 837.
25. Siegbahn, K. (1970) *Philos. Trans. R. Soc. London,* **A 268** : 33.
26. Carver, J.C., Schweitzzer, A.K. & Carlson, T.A. (1972) *J. Chem. Phys.* **57** : 973.
27. Shrivastava, B.D., Joshi, S.K. & Pandeya, K.B. (1988) *X-ray Spectrom.* **17** : 127.
28. Cauchois, Y. & Senemaud, C. (1978) *Wavelengths of X-ray Emission lines and Absorption Edges,* Pergamon Press, New York.
29. Gianturco, F.A. & Coulson, C.A. (1968) *Mol. Phys.* **14** : 223.
30. Clementi, E. (1965) *IBM J. Res. Dev. Suppl.* **9** : 2.
31. Meisel, A. (1965) *Phys. Stat. Solidi,* **10** : 365.
32. Verma, L.P. & Agarwal, B.K. (1968) *Ind. J. Pure and Appl. Phys.* **5** : 616.
33. Verma, L.P. & Agarwal, B.K. (1968) *J. Phys. C.* 11658.
34. Yeh, H.C. & Azaroff, L.V. (1967) *J. Appl. Phys.* **38** : 4034.
35. Kondawar, V.K. & Mande, C. (1976) *X-ray Spectrom.* **5** : 2.
36. Hemchandran, K. & Chetal, A.R. (1986) *Phys. Stat. Sol. (b)* **136** : 181.
37. Shrivastava, B.D., Kumawat, R.C., Joshi, S.K. & Bhattacharya, P.K. (1993) *J. Ind. Chem. Soc.* **70** : 731.
38. Patel, R.N. & Pandeya, K.B. (1997) *Transition Met. Chem.* **22** : 132.
39. Suchet, J.P. (1965) *Ann. Chem.* **10** : 517.
40. Ovsannikova, I.A., Batsanova, S.S., Nasanova, L.I. & Nekrasova, E.A. (1967) *Bull. Acad. Sci., USSR* **31** : 936.

41. Khadikar, P.V., Mangelson, N.F. & Pandharkar, S.P. (1989) *Jpn. J. Appl. Phys.* **28** : 709.

42. Stern, E.A., Sayers, D.E. & Lytle, F.W. (1975) *Phys. Rev.* **B 11** : 4836.

43. Lytle, F.W., Sayers, D.E. & Stern, E.A. (1975) *Phys. Rev.* **B 11** : 4825.

44. Shulman, R.G., Eisenberger, P. & Blumberg, W.E. (1975) *Proc. Natt. Acad. Sci., USA,* **72** : 4003.

45. Lee, P.A. & Beni, G. (1979) *Phys. Rev.* **B 15** : 2862.

46. Levy, R.M. (1965) *J. Chem. Phys.* **43** : 1846.

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Chromosome preparations from freshly dead fish

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Abstract Chromosome preparations were obtained from kidney tissues of freshly dead fish *Labeo rohita* and *Cirrhinus mrigala*, using *in vitro* colchicine treatment. The chromosomal spreads were of good quality, suitable for karyotyping and chromosome banding studies. The present technique is highly suitable for cytogenetic studies in wild stock of fish species for which there is difficulty in getting live fish and also in those species which die immediately in captivity. This is the first report on use of tissues from dead fish for studying metaphase chromosomes.

(**Keywords**: chromosome/*Labeo rohita/cirrhinus mrigala*)

Chromosome spreads in fish have been commonly obtained from tissues such as gill, kidney, intestine and blood by using techniques such as 1) *in vivo* colchicine pretreatment and sacrificing fish specimens[1-3]. 2) cell culture techniques[4-6]. 3) *in vitro* colchicinization technique[7,8]. All these techniques require live fish for chromosome preparations which sometimes can be very difficult especially under field conditions. So far no report is available in literature on use of tissues from dead fish for obtaining chromosomes and it is widely believed that dead fish can not be used for chromosomal studies. The present investigation was therefore, undertaken to explore the possibility of using tissues from freshly dead fish for karyomorphological studies.

For the present study, five specimens of two species i.e., *Labeo rohita* and *Cirrhinus mrigala*, approximately 0.5 to 1 kg in weight were procured from the local market in the month of December. The kidney tissues were dissected out from dead specimens, approximatley 2 hr. after death. The cephalic kidney was taken and homogenized to prepare cell suspension in 8ml of RPMI 1640 culture medium. The cell suspension was taken in a petridish and to this cell suspension 50 µl of 0.05% of colchicine was added. The cell suspension was incubated at 37°C for 30 minutes and then the contents in petridish were centrifuged at 1200 rpm for 10 minutes. The cell pellet was suspended in 8 ml of freshly prepared 0.56% potassium chloride solution for 22 minutes at room temperature. The hypotonic treatment was terminated by adding 1ml of freshly prepared, chilled Carnoy's fixative (Methanol 3 parts: acetic acid 1 part) to the centrifuge tube. After thoroughly mixing the contents the tubes were again centrifuged at 1200 rpm for 10 minutes. The supernatant was discarded and the cell

pellet was resuspended in 7 ml of chilled fixative. The process of washing of the cell pellet with fixative was repeated thrice to get clear whitish pellet. The slides were prepared by the flame drying technique and stained with Giemsa in phosphate buffer (pH 6.8).

More than 25 good quality metaphase spreads were observed in each slide from all dead specimens. The diploid chromosome number in both the species was 2n= 50 (Figs. 1&2), which was in agreement with the chromosome number, reported earlier[9-10]. The quality of metaphase spreads obtained was suitable for karyotyping and chromosome banding studies. The number of metaphase spreads obtained in the present study was generally sufficient for cytogenetic characterization in these species.

For cytogenetic studies of fishes, the *in vivo* techniques are commonly used and optimized in different species to get a large number of metaphase spreads. This method has, however, a disadvantage that specimens have to be kept alive at least for one hour after injecting colchicine. In field conditions it is very difficult to get and maintain live specimens. In some species like *Tenuolosa ilisha* the fishes die immediatley in captivity[11]. Similarly setting up of cell cultures in these species would be very difficult. At present cell culture techniques have been reported in some carps and trout only[4,6,12]. The difficulty in getting live fishes may be one of the reasons for lack of karyological information in many fish species especially wild species.

Thus, with the technique discussed above, cytogenetic investigation could be undertaken in wild stocks of freshly dead fishes under field conditions obtained from routine catches at landing centers. This technique has an additional advantage that cytogenetic studies can be undertaken along with biochemical and molecular genetics work as an integrated study leading to better interpretation of results. This is the first report on chromosomal preparations from tissues of freshly dead fishes.

References

1. Gold, J.R. (1974) *Prog. Fish. Cult.* **36** : (3) 169.
2. Klingerman, A.D. & Bloom, S.E. (1977) *J. Fish. Res. Board. Can.* **34** : 266.
3. Almeida-Toledo, L.F., Bigoni, A.P.V. (1995) *Aquaculture* **135** : 227.
4. Hartley, S.E. & Horne, M.T. (1983) *J. Fish. Biol.* **22** : 77.
5. Blaxhall, P.C. (1983) *J. Fish. Biol.* **22** : 61.
6. Sathe, P.S. Mourya, D.T., Basu, A., Gogate, S.S. & Banerjee, K. (1995). *Ind. J. Exp. Biol.* **33** : 589.
7. Foresti, F., Oliveria, C. & de Almeidae Toledo L.F. (1993) *Experientia* **19** : 810.
8. Nagpure, N.S. & Barat, A. (1997) *Ind. J. Exp. Biol.* **35** : 915.
9. John, G., Barat, A. & Lakra, W.S. (1993) *La Kromosomo.* II **70** : 2381.
10. Khuda, Bukhsh, A.R. & Chakrabarty, C. (1994) *J Inland Fish Soc India* **26**(1) : 44.
11. Barat, A., Punia, P. & Ponniah, A.G., (1996) La *Kromosomo.II* **82** : 2828.
12. Sanchez, L., Martinez, P., Vinas, A. & Bouza, C. (1990) *Cytogenet. Cell. Genet.* **54** : 6.

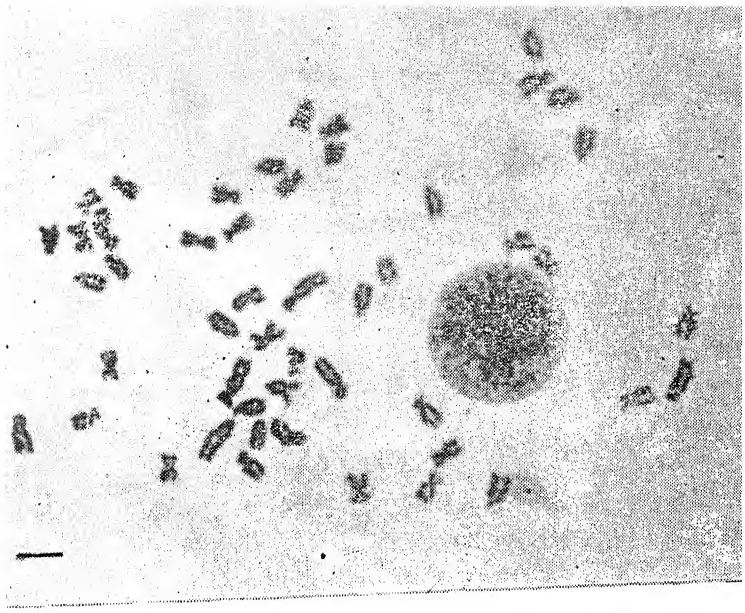

Fig. 1 Metaphase spread of *L. rohita*

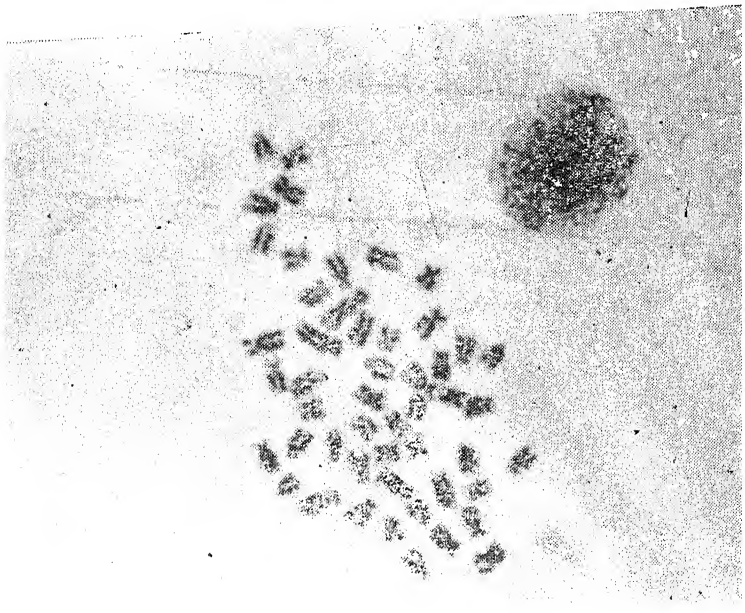

Fig. 2 Metaphase spread of *C. mrigala*
Bar represents 10μm

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Bait shyness in *Mus musculus* albino to difethialone

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Abstract Bait shyness and poison aversion studies were conducted in the laboratory against *Mus musculus* albino. Poison bait intake was not significantly different in both preferred and less preferred food. Poison baits were equally palatable and bait shyness was not observed. Difethialone 0.000625% shows no sickness in animals.

(**Keywords :** *Mus msuculus*/difethialone/toxicity)

The losses due to rodent pest during crop production, processing, storage and transport are imprecise and conservative. The most common and effective means to control these pests, is the use of rodenticides. The main hazard in controlling the rodent pest with poison is the development of bait shyness. A number of rodent species while feeding upon these poison baits take only a sublethal dose of the poison and recover after withstanding a period of sickness. The development of bait shyness and poison aversion amongst rodents as a result of rapid onset of poisoning symptoms before ingestion of lethal dose of poisons, rendered the acute single dose rodenticides relatively ineffective[1]. The behavioural aspects of bait shyness and poison aversion have been studied by several workers[2,3,4].

Difethialone is highly potent single feed newly introduced second generation anticoagulant rodenticide. Therefore, present study was carried out in the laboratory against *Mus musculus* albino.

Poison aversion and bait shyness behaviour in *Mus musculus* albino was evaluated in mice of both the sexes by feeding difethialone bait. For each trial acclimatized healthy twelve mice of almost equal body weight and of both the sexes were used from the maintained colony. Experiment was completed in two phases.

Phase A : Twelve individually caged healthy mice (6 males and 6 females) were taken for this experiment. Animals were provided with the most preferred bajra flour and second preferred wheat flour baits without poison at a time for three days. Water was available *ad libitum.*

Phase B : From the fourth day poison bait at 0.000625% concentration was also given to the animals of phase A along with the non-rodenticidal bait of less preferred flour (wheat). This bait was given upto seventh day. Water was available *ad libitum.* Difference in the consumption of two baits were analyzed statistically.

Results of the investigations are presented in Table 1. It is evident from Table 1 that the mean daily intake of bajra was more (Students t test, $p < 0.05$) than wheat.

Table 1 – Bait shyness test with Difethialone (0.000625%) in Albino Mice

Phase A

Carrier Food	Mean Daily Intake of Food (g./100 gm b.w.t.)			Mean of Total Daily Intake (g/100 gm b.wt.)
	1st day	2nd day	3rd day	
Bajra	14.85±3.85	15.93±4.55	10.52±1.64	13.77±1.65
Wheat	9.21±4.30	7.38±2.66	10.44±3.90	9.01±0.88
				(P < 0.05)

Phase B

Carrier Food	Mean Daily Intake of Food (g/100gm b.wt.)				Mean of Total Daily Intake
	4th day	5th day	6th day	7th day	(g/100 gm.b.wt.)
Bajra+2% salt +2% coconut oil +0.000625% difethialone	11.85±3.01	15.81±2.72	15.31±2.87	12.45±2.46	13.85±0.00
Wheat	8.85±4.95	8.48±4.09	7.97±3.06	8.90±3.38	8.55±0.21
				(P < 0.05)	(P < 0.01)

In phase B (4th day) the consumption of bajra decreased when difethialone was mixed with this flour but the intake of bajra (poisoned bait) was still higher than that of wheat (plain bait) (P>0.05). On 5th and 6th day (2nd and 3rd day of poison baiting) the consumption of bajra increased again. It was more than plain bait (P>0.05). On 7th day (4th day of poison baiting) the total intake was reduced due to sickness but it was still higher (P<0.05) than less preferred (poison free) plain bait. Significant difference (P<0.01) between mean of total daily intake of most preferred flour (poison bait) and less preferred flour (poison free) indicates absence of bait shyness and good acceptability of the rodenticide.

There is however no positive evidence that anticoagulant poisons induce bait shyness in field and laboratory. Such observations have already been reported for various rodenticides in a number of rodent species[8,9,10,11,12].

It is, therefore, recommended that if poison baiting with difethialone is continued for more than one day it can successfully control field and commensal rodents and suppress the bait shy population after the treatment of acute poisons.

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References

1. Barnett, S.A. (1948) *Agriculture Studies*, F.A.O. Rome **2** : 129.
2. Ojha, P. (1978) *Rodent Newsl.*, ICAR **2(1)** : 2.
3. Rana, B.D. (1980) *Annals of Arid Zone*, **19(4)** : 511.
4. Soni, G.R. & Prakash, I. (1988) *Ind. Jour. of Exp. Biol.*, **26** : 476.
5. Boyle, C.M. (1960) *Nature*, **188** : 517.
6. Jackson, W.B., Brooks, J.E., Bowerman, A.M. & Kaukeinen, D.E. (1975) *Pest Control*, **43(5)** : 14, 16, 18, 20, 22, 24.
7. Arora, K.K. & Lal, S.S. (1979) *Indian J. Rodent* **1** : 24.
8. Prakash, I. & Jain, A.P. (1971) *Ann. Appl. Biol.*, **69** : 169.
9. Saxena, Y. & Sharma, R.K. (1982) *Bull. Grain Tec.* **20(1)** : 43.
10. Rao, A.M.K.M. & Prakash, I. (1980) *Indian J. Exp. Biol.* **18(12)** : 1490.
11. Kumar, D. (1990) *Rodent Newsl.* **14** : 8.
12. Singh, R. & Saxena, Y. (1991) *Rodent Newsl.* **15** : 7.

Science News

Origin and Control of Pandemic Influenza

High virulence of the flu virus that caused the "Spanish influenza" pandemic of 1918 in which more than 20 million people died, has been reanalysed by the Gibbs *et al.* The authors held that the hemagglutinin gene which is a key virulence determinant originated by recombination and the recombination event occured at about the same time as the spanish flue pandemic started.

What can be done if a new influenza virus suddenly appears and spreads with alarming speed around the world? Development of the vaccines is a slow process and requires time. In the situation antiviral neuraminidase inhibitors Relenza and Tamiflu might provide the first line of defense against the new flue virus. Neuraminidase one of the glycoprotein "spikes" on the surface of the influenza virus – is an enzyme that cleaves sialic acid residues from receptors for the virus enabling the virus to spread throughout the body. Inhibition of this enzyme stops this spread and effectively curtails the infection.

According to the report on virology in perspective it is suggested that for effective control measures the stocking of the above mentioned drugs are necessary and the investments made by governments would be worthwhile.

Courtesy : Science, 2001

Risk of Vitamin C induced Cancer

Paracelsus (1493-1541) a Swiss philosopher and lecturer in medicine stated that all substances are poisons, there is none which is not a poison. The right dose differentiates a poison and remedy. In sixteenth century, the paracelsus's view, to a greater extent was based on his own experiences rather than being based on proper experimental planning, now it finds new strength with the recent scientific reports on vitamin C from university of Pennsylvania and University of Brighton that the vitamin C overdosing may lead to the development of cancer. A dose of 2 gms daily intake is proved to be highly damaging to the DNA which in turn may undergo defective replication.

Vitamin C is basically known to act as a physiological modulator. Its capacity to combat free radicals which cause aging, is doubtlessly significant. Following the overdosing, the beneficial or medicinal action of the vitamin may undergo reversal by breaking down natural fatty acids into potent toxicants within the body systems and ultimately consequencing serious health hazards.

In Britain the allowed daily intake dose is 30 mg. while in India 250 and 500 mg tablets are available in markets. Most of the medical practitioners in general are prescribing at least single tablet dose daily in physiological disorders and infections. The fact raises a very pertinent question – whether Indians are at an increased risk of vitamin C induced cancer in the 21st century?

In fact the situation probably is not so worse. The vitamin is capable of causing

cancer, is based on the physical and chemical studies of isolated DNA with qualitative concerns. However, the qualitative studies of DNA in intact biological systems are yet to be established. However, a holistic approach towards the risk assessment of vitamin C in biological systems is being suggested rather than adopting the reductionist approach. And after all Paracelsus's view underlines the need of wise use of the vitamin.

Courtesy : New Scientist
Summarised by Dr. U.P. Rai

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